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SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF NOVEL ISOXAZOLE BEARING PYRAZOLE DERIVATIVES FOR ANTIBACTERIAL ACTIVITY

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Abstract:

Objective: *The objective of the paper was to synthesis and characterization of new isoxazole bearing pyrazole derivatives and evaluate them for antibacterial activity.*

Material and methods: *The isoxazole bearing pyrazole derivatives have been synthesized by the utilize the four-step process. Total Ten compounds have been synthesized and characterized by IR, [1]HNMR, elemental and mass spectral analysis. The final compounds (PIA-1 to PIA-10) have been evaluated for antibacterial activity by disk diffusion method using sulfamethoxazole as standard drug.*

Result and Discussion: *The IR spectra of compound PIA-1 to PIA-10 shown the characteristics peak at 3052 (N-H), 2964 (C-H), 1628 (C=O), 1448 (C=N), 1625 (C=C), 1126 (C-O); 1015 (C-Br); 850 (C-Cl) and 1102 (C-F). The 1HNMR spectrum show the characteristics peak δ 6.68-6.70 (d, 2H, Ar-Fluorine), 7.14-7.16 (d, 1H, Ar-chlorine), 7.28-7.30 (m, 5H, Ar-H), 7.58-8.00 (d, 2H, Ar-Chlorine), 7.75-7.78 (d, 2H, Ar-Fluorine), 8.25 (s, 1H, Ar-H isoxazole), 10.97 (s, 1H, -Ar-H pyrazole); 7.26 (s, 1H, Ar-Cl). The antibacterial evaluation of the synthesized compounds stated that Isoxazole-pyrazole derivatives exhibit considerable inhibitory effect on all bacteria strains at both 50 µg/ml and 100 µg/ml dosing levels. PIA-1, PIA-2, PIA-3, PIA-4 had the highest activity in gram positive bacterial stains and PIA-1, PIA-3, PIA-4 and PIA-10 have shown best activity against gram negative bacteria.*

Conclusion*: The antibacterial activity of the synthesized compounds (PIA-1, PIA-3, PIA-4 and PIA-5) was significantly similar or better than that of standard Drug used for comparison. These Isoxazole bearing pyrazole compounds have demonstrated potency as a superior antibacterial agent, although more in-vivo research on the efficacy and dosage regimen of the compounds is needed.*

Keywords: *Antibacterial activity, Pyrazole, Isoxazole, sulfamethoxazole, disk diffusion*

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INTRODUCTION:

The discovery of new antimicrobial compounds is a major goal in the context of today's COVID-19 pandemic. It is known that both patients with severe cases and patients with moderate cases of COVID-19, with or without pneumonia, have received treatments with various antibiotics.[1]

Isoxazoles are an important class of five membered ring system, displaying a wide variety of biological properties including antiviral, antidepressant and anti-inflammatory activities.[2] In fact, the isoxazole possess significant synthetic applications, diverse biological properties and represent a unique class of pharmacophore present in many therapeutic agents. Also, they showed, anti-tubercular, antifungal and anti-influenza effects. On the other hand, isoxazole structural motif is found in the COX II inhibitors and parecoxib because of its capability to exhibit a wide range of bioactivities, including the anti-inflammatory and antimicrobial activities.[3]

Pyrazole are unique in their chemical behaviour not only among heterocyclic compounds in general, but also among related azoles.**[4]** This is because pyrazole possesses the typical properties of the aromatic system, which are in fact rather pronounced in these derivatives, together with the high liability of the ring under certain condition. Marketed products of pyrazole includes Celecoxib**[5],** Phenzone, (phenazon or antipyrine), Lonazolac[**6**], Rimonabant, Fipronil, Tepoxalin, Fomepizole or 4-methylpyrazole is indicated for use as an antidote in confirmed or suspected methanol or ethylene glycol poisoning.[7]

The literature survey revealed that pyrazole derivatives have been extensively studied but to the best of our knowledge there is no report of any incorporating an isoxazole moiety. Taking into consideration, various biological activities of isoxazole cited in the literature, it appeared to us interesting to think about to combine these two moieties with hope to access to more biologically effective compounds.

The objective of the paper was to design, synthesis, characterization and biological evaluation of new Isoxazole bearing pyrazole derivatives and evaluate for antibacterial potential by disk diffusion method.

MATERIAL AND METHOD:

The 1-(3,5-dichloro-2-fluorophenyl) ethan-1-one, methanol, phenyl hydrazine and acetic acid was purchased from Merck, India. The dimethyl formamide and phosphorus oxy chloride, Isopropyl alcohol, potassium hydroxide, ethanol and Hydroxylamine hydrochloride was purchased from sigma Aldrich. All the chemicals were purchased from Merck India. Commercial grade solvents used for the reactions were distilled before use. The melting points of the synthesized compounds were determined in open glass capillaries. IR spectra were recorded on Bruker-alpha FTIR spectrometer. Elemental analysis was performed and found values were within 0.4% of theoretical values. 1HNMR spectra were recorded at 400 MHz, Mass Spectra were recorded using Mass Spectrometers Jeol FSX-112 (FAB) by ESI.

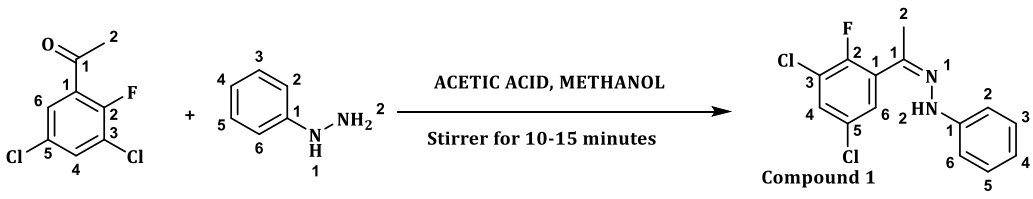
Chemistry:

Synthetic strategy planning for synthesis of titled compound "5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-substituted)isoxazole"

The synthesis was divided into four Scheme:

Scheme-I: Synthesis of compound (Z)-1-(1(3,5-dichloro-2-fluorophenyl)ethylidene-2-phenyl hydrazine; Scheme-II: Synthesis of 5-(2,4-dichloro-5-fluorophenyl)-2-phenyl-3H-2l4-pyrazole-4carb-aldehyde; Scheme-III: Synthesis of (E)-3-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-(p-substituted)prop-2-en-1-one and Scheme-IV:- Synthesis of 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-substituted) isoxazole

Scheme-I: Synthesis of compound (Z)-1-(1(3,5-dichloro-2-fluorophenyl)ethylidene-2-phenyl hydrazine



1-(3,5-dichloro-2-fluorophenyl)ethan-1-one

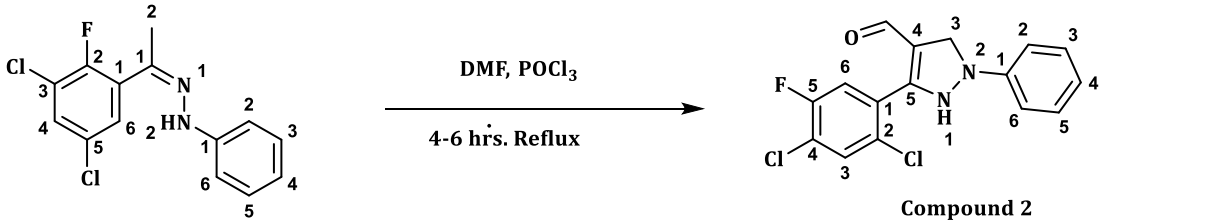
phenylhydrazine

(*Z*)-1-(1-(3,5-dichloro-2-fluorophenyl)ethylidene)-2-phenylhydrazine

Procedure: The 1-(3,5-dichloro-2-fluorophenyl)ethan-1-one (0.02 M) is dissolved in

methanol (10 ml) and phenyl hydrazine (0.02M) was dissolved in acetic acid (15 ml). Both the solution was mixed in the round bottom flask and stirred for 10-15 minutes at 200 rpm at 25-30˚C.[8] The reaction was monitored by the TLC method using the ethyl acetate and methanol (4:2) as solvent system. When the reaction is completed, the mixture poured into ice cold distilled water, the solid product obtained was filtered and washed several times with ice cold water. The compound 1, obtained as solid product was recrystallized with ethanol: water (4:2).

Scheme-II: Synthesis of 5-(2,4-dichloro-5-fluorophenyl)-2-phenyl-3H-2l4-pyrazole-4-carb aldehyde



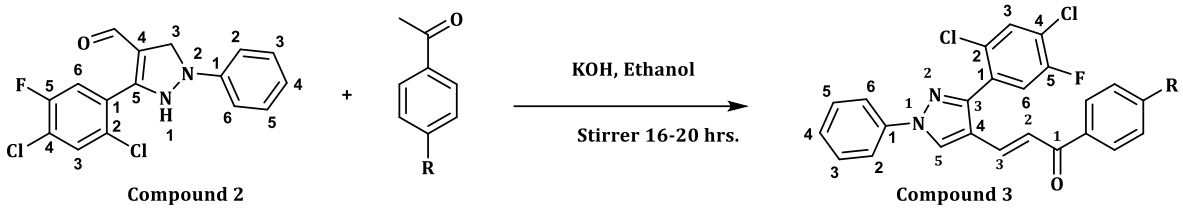
Compound 1

Compound 2

5-(2,4-dichloro-5-fluorophenyl)-2-phenyl-3H-2l4-pyrazole-4-carbaldehyde

Procedure: The compound 1, (0.01M) was dissolved in dimethyl formamide and equimolar quantity of $POCl_3$ was mixed in it and reflux for 4-6 hrs. at 75˚C. The reaction was monitored by TLC method using methanol: ethyl acetate as solvent system. After the compilation of reaction take 30 min at room temperature. The reaction mixture was poured into crushed ice followed by neutralization using sodium bicarbonate.[9] The product was recrystallised from methanol.

Scheme-III: Synthesis of (E)-3-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-(p-substituted)prop-2-en-1-one



Compound 2

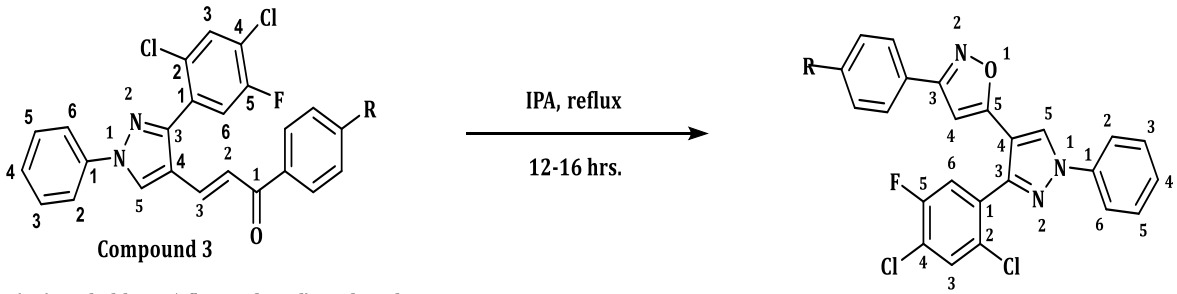
5-(2,4-dichloro-5-fluorophenyl)-2-phenyl-3H-2l4-pyrazole-4-carbaldehyde

Compound 3

(*E*)-3-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-(*p*-substituted)prop-2-en-1-one

Procedure: The compound 3, (0.02M) and 1-(4-susbstitutedphenyl)ethan-1-one derivatives (0.02M) was dissolved in ethanolic potassium hydroxide (basic medium) and stirrer for 16-20 hrs. After the completion of reaction, mixture was poured into crushed ice. [10] The solid product was filtered and washed with ice-cold water.

Scheme-IV: Synthesis of 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-substituted)isoxazole



Compound 3

(*E*)-3-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-(*p*-substituted)prop-2-en-1-one

5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(*p*-substituted)isoxazole

Procedure: The different substituted compound 3, (0.02M) was dissolved in Isopropyl alcohol (30 ml) and Hydroxylamine hydrochloride (0.02M) and sodium acetate (0.02M) was mixed and refluxed for 12-16 hrs. The reaction was monitored by TLC technique and after completion of reaction cool to room temperature and pour onto the ice-cold water. The obtained solid product was filtered and washed several times with distilled water and recrystallized by methanol. [11]

PHARMACOLOGICAL SCREENING:

Antibacterial activity:

The Ten Isoxazole bearing pyrazole compounds naming as PIA-1 to PIA-10 were synthesized and evaluated for the antimicrobial activity i.e., antibacterial activity. In antibacterial screening of compounds, both gram positive and gram-negative strains were utilized in that case of Gram-positive strains (*Bacillus subtilis* and *Micrococlus luteus*); Gram negative Organisms (*Pseudomonas aeruginosa* and *Escherichia coli*) were used respectively. Disc diffusion method is applied for the determination of zone of inhibition. Sulfamethoxazole is used as standard drug for the antibacterial activity. [12]

Disk diffusion method

The antibacterial activity of the synthesized compounds determined by the dissolving the tested compound (PIA-1 to PIA-10) in phosphate buffer saline at the concentration of 50 and 100µg/ml against Muller Hinton agar medium by disc diffusion method.[13] Muller Hinton agar is considered the best medium to use for routine susceptibility testing of non-fastidious bacteria and it shows acceptable batch-to-batch reproducibility for susceptibility testing.[14] All the ingredients were taken in a conical flask and dissolved in 1000 ml of distilled water and heated in a steam bath to dissolve. The pH was adjusted to 7.0±0.2 and sterilized in autoclave at 15lb pressure at 120˚C for 15 min. The sterile medium was poured into petri dish and allowed to solidify. What-man filter paper grade-1 disc of 5 mm diameter and 2 mm width was sterilized by autoclaving for 15 min at 121°C.[15]

The sterile discs were impregnated with different synthesized compounds which were dissolved in dimethyl form. The sterilized agar petri-dish was seeded with test bacteria and the impregnated discs were placed on the medium with suitable space between the discs. The plates were incubated at 37±1°C for 18-24 hrs. for bacterial medium. The inhibition of zones caused by the synthesized compounds and standard drug were examined and the diameter of zone of inhibition was observed and recorded.[16]

RESULT AND DISCUSSION:

Spectral analysis:

The synthesis of the compounds was divided in Four scheme. In Scheme-I, **Compound 1,** (Z)-1-(1(3,5-dichloro-2-fluorophenyl)ethylidene-2-phenyl hydrazine was synthesized by reaction of 1-(3,5-dichloro-2-fluorophenyl)ethan-1-one and phenyl hydrazine and characterized by the IR, 1HNMR and Mass spectral analysis and spectral data stated that white crystal of the compound having the melting point 120-122°C. The IR spectrum has shown the characteristics peak at 3345 (NH), 3120 (CH aromatic), 852 (C-Cl); 1015 (C-Br), 1102 (C-F). The 1HNMR spectrum shown the peak of NH (9.82), (Ar-H) 6.82–6.85. The Mass spectrum has shown the peak at 297.12, which is confirms the molecular weight of compounds.

In Scheme-II, **Compound 2** (5-(2,4-dichloro-5-fluorophenyl)-2-phenyl-3H-2l4-pyrazole-4-carbaldehyde) was synthesized by the reaction of compound 1 with DMF and $POCl_3$. The compound 2 obtained as solid white crystals having yield 86% with melting point 160-162°C. The FT-IR spectrum shown the peak at 3296 (NH), 3120 (CH Aromatic), 2950 (CH aliphatic), 1688 (C=O); 850 (C-Cl); 1018 (C-Br); 1112 (C-F). The 1HNMR spectra show the peak at 8.53 (NH), 3.00–2.90 (m, 4H) and 13C NMR spectrum has shown the peak at 164.3 (C=O), 114-129.1 (4, CH), 66.1 (CH_2), 46.0 (2, CH_2), 42.8 (2, CH_2). In scheme-III, **compound 3** [(E)-3-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-(p-substituted)prop-2-en-1-one] was synthesized.

In Scheme-IV **compound 4,** 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-substituted)isoxazole (PIA-1 to PIA-2) was synthesized by the reaction of Compound 3, (E)-3-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1-(p-substituted)prop-2-en-1- one) reacted with Hydroxylamine hydrochloride (0.02M) and sodium acetate (0.02M) in presence of Isopropyl alcohol and reflux up to 12-16 hrs. to synthesized compound 4, (5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-substituted)isoxazole) the acidic condition to 60-82% yields (**Scheme-4**).

Ten compounds of isoxazole bearing pyrazole derivatives were produced and analyzed using IR, 1HNMR, mass spectral, and elemental analysis. The Final compounds PIA-1 to PIA-10 was synthesized and characterized for physicochemical properties. The compound PIA-1 having the Chemical formula $C_{24}H_{13}Cl_3FN_3O$, Mol. Weight 484.74, Percentage

yield 78%, melting point 225-227°C and Rf value is 0.42. The IR spectra of compound PIA-1 to PIA-10 revealed the following features: 3052 (N-H), 2964 (C-H), 1628 (C=O), 1448 (C=N), 1625 (C=C), 1126 (C-O); 1015 (C-Br); 850 (C-Cl) and 1102 (C-F). The 1HNMR spectrum of Compound PIA-1 to PIA-10 shown the characteristics peak *δ* 6.68-6.70 (d, 2H, Ar-Fluorine), 7.14-7.16 (d, 1H, Ar-chlorine), 7.28-7.30 (m, 5H, Ar-H), 7.58-8.00 (d, 2H, Ar-Chlorine), 7.75-7.78 (d, 2H, Ar-Fluorine), 8.25 (s, 1H, Ar-H isoxazole), 10.97 (s, 1H, -Ar-H pyrazole)*;* 7.26 (s, 1H, Ar-Cl). The mass spectrum of all sixteen compounds was recorded, and it was virtually identical to the molecular weight of the compounds. The compound's elemental analysis and melting point were also recorded and evaluated, and both were within acceptable limits.

PIA-1: 3-(4-chlorophenyl)-5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)isoxazole
Chemical formula: $C_{24}H_{13}Cl_3FN_3O$; Molecular weight: 484.74; IR (cm^{-1}): 3052 (N-H) 2964 (C-H); 1628 (C=O); 1448 (C=N); 1625 (C=C); 1126 (C-O); 1015 (C-Br); 850 (C-Cl)
1102 (C-F); 1HNMR (ppm): δ6.68-6.70 (d, 2H, Ar-Fluorine), 7.14-7.16 (d, 1H, Ar-chlorine), 7.28-7.30 (m, 5H, Ar-H), 7.58-7.62 (d, 2H, Ar-Chlorine), 7.75-7.78 (d, 2H, Ar-Fluorine), 8.25 (s, 1H, Ar-H isoxazole), 10.97 (s, 1H, -Ar-H pyrazole); 7.26 (s, 1H, Ar-Cl); FAB Mass (m/z): 484.50.

PIA-2: 3-(4-bromophenyl)-5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)isoxazole
Chemical Formula: $C_{24}H_{13}BrCl_2FN_3O$; Molecular weight: 529.19; IR (cm^{-1}): 3050 (N-H);
2965 (C-H); 1620 (C=O); 1445 (C=N); 1620 (C=C); 1121 (C-O);1012 (C-Br); 848 (C-Cl);
1098 (C-F); 1HNMR (ppm): δ 6.68-6.72 (d, 2H, Ar-Fluorine), 7.15-7.17 (d, 1H, Ar-chlorine), 7.28-7.30 (m, 5H, Ar-H), 7.-7.58 (d, 2H, Ar-chlorine), 7.74-7.75 (d, 2H, Ar-Fluorine), 8.23 (s, 1H, Ar-H isoxazole ring), 10.97 (s, 1H, -Ar-H pyrazole ring); FAB Mass (m/z): 529.17

PIA-3: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-fluoro phenyl)isoxazole
Chemical Formula: $C_{24}H_{13}Cl_2F_2N_3O$; Molecular weight: 468.28; IR (cm^{-1}): 3055 (N-H); 2968 (C-H); 1622 (C=O); 1440 (C=N); 1622 (C=C); 1124 (C-O); 1015 (C-Br); 845 (C-Cl); 1108 (C-F); 1HNMR (ppm): δ 6.64-6.66 (d, 2H, Ar-Fluorine), 7.12-7.14 (d, 1H, Ar-Chlorine), 7.25-7.32 (m, 5H, Ar-H), 7.57-7.58 (d, 2H, Ar-chlorine), 7.82-7.84 (d, 2H, Ar-Fluorine), 8.15 (s, 1H, Ar-H isoxazole ring), 10.97 (s, 1H, -Ar-H pyrazole ring). FAB Mass (m/z): 468.10.

PIA-4: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-nitrophenyl) isoxazole
Chemical Formula: $C_{24}H_{13}Cl_2FN_4O_3$; Molecular weight: 495.29; IR (cm^{-1}): 3050 (N-H); 2962 (C-H); 1625 (C=O); 1442 (C=N); 1628 (C=C); 1120 (C-O); 1014 (C-Br); 848 (C-Cl); 1112 (C-F); 1350 (N-O);1562 (N=O);1HNMR (ppm): δ 6.62-6.68 (d, 2H, Ar-Fluorine), 7.15-7.18 (d, 1H, Ar-Chlorine), 7.28-7.30 (m, 5H, Ar-H), 7.55-7.58 (d, 2H, Ar-Chlorine), 7.74-7.75 (d, 2H, Ar-Fluorine), 8.26 (s, 1H, Ar-H isoxazole ring), 10.95 (s, 1H, -Ar-H pyrazole ring). FAB Mass (m/z): 495.20

PIA-5: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-tolyl) isoxazole
Chemical Formula: $C_{25}H_{16}Cl_2FN_3O$; Molecular weight: 464.32; IR (cm^{-1}): 3050 (N-H); 2962 (C-H); 1625 (C=O); 1442 (C=N); 1620 (C=C); 1124 (C-O); 1012 (C-Br); 848 (C-Cl); 1112 (C-F); 1HNMR (ppm):δ 6.62-6.64 (d, 2H, Ar-F), 7.13-7.15 (d, 1H, Ar-Cl2), 7.25-7.30 (m, 5H, Ar-H), 7.55-7.59 (d, 2H, Ar-Cl2), 7.72-7.78 (d, 2H, Ar-F), 8.12 (s, 1H, Ar-H isoxazole ring), 10.93 (s, 1H, -Ar-H pyrazole ring); FAB Mass (m/z): 464.25.

PIA-6: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-methoxyphenyl) isoxazole
Chemical Formula: $C_{25}H_{16}Cl_2FN_3O_2$; Molecular weight: 480.32; IR (cm^{-1}): 3050 (N-H); 2962 (C-H); 1624 (C=O); 1442 (C=N); 1624 (C=C); 1128 (C-O); 1012 (C-Br); 848 (C-Cl); 1098 (C-F); 1HNMR (ppm): δ 6.68-6.70 (d, 2H, Ar-Fluorine), 7.12-7.14 (d, 1H, Ar-Chlorine), 7.28-7.34 (m, 5H, Ar-H), 7.59-8.02 (d, 2H, Ar-Chlorine), 7.78-7.82 (d, 2H, Ar-Fluorine), 8.21 (s, 1H, Ar-H isoxazole ring), 10.96 (s, 1H, -Ar-H pyrazole ring); FAB Mass (m/z): 480.30.

PIA-7: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-ethyl phenyl) isoxazole
Chemical Formula: $C_{26}H_{18}Cl_2FN_3O$; Molecular weight: 478.35; IR (cm^{-1}): 3050 (N-H); 2962 (C-H); 1625 (C=O); 1446 (C=N); 1620 (C=C); 1120 (C-O); 1012 (C-Br); 846 (C-Cl); 1108 (C-F);1HNMR (ppm): δ6.62-6.64 (d, 2H, Ar-Fluorine), 7.15-7.18 (d, 1H, Ar-Chlorine), 7.25-7.32 (m, 5H, Ar-H), 7.57-7.58 (d, 2H, Ar-Chlorine), 7.74-7.75 (d, 2H, Ar-F), 8.15 (s, 1H, Ar-H isoxazole ring), 10.91 (s, 1H, -Ar-H pyrazole ring); FAB Mass (m/z): 478.20.

PIA-8: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-ethoxy phenyl) isoxazole

Chemical Formula: $C_{26}H_{18}Cl_2FN_3O_2$; Molecular weight: 494.35; IR (cm^{-1}): 3050 (N-H); 2962 (C-H); 1625 (C=O); 1450 (C=N); 1622 (C=C); 1124 (C-O); 1012 (C-Br); 848 (C-Cl);1106 (C-F); 1HNMR (ppm): δ 6.66-6.68 (d, 2H, Ar-Fluorine), 7.10-7.12 (d, 1H, Ar-Chlorine), 7.28-7.34 (m, 5H, Ar-H), 7.56-7.58 (d, 2H, Ar-Chlorine), 7.72-7.74 (d, 2H, Ar-Fluorine), 8.21 (s, 1H, Ar-H isoxazole ring), 10.93 (s, 1H, -Ar-H pyrazole ring). FAB Mass (m/z): 494.20.

PIA-9: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-propyl phenyl) isoxazole

Chemical Formula: $C_{27}H_{20}Cl_2FN_3O$; Molecular weight: 492.38; IR (cm^{-1}): 3045 (N-H); 2960 (C-H); 1625 (C=O); 1442 (C=N); 1620 (C=C); 1121 (C-O); 1012 (C-Br); 849 (C-Cl); 1108 (C-F); 1HNMR (ppm): δ 6.62-6.64 (d, 2H, Ar-Fluorine), 7.10-7.12 (d, 1H, Ar-Chlorine), 7.25-7.32 (m, 5H, Ar-H), 7.57-7.58 (d, 2H, Ar-Chlorine), 7.74-7.75 (d, 2H, Ar-Fluorine), 8.15 (s, 1H, Ar-H isoxazole ring), 10.91 (s, 1H, Ar-H pyrazole ring); FAB Mass (m/z): 492.30.

PIA-10: 5-(3-(2,4-dichloro-5-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-propoxy phenyl) isoxazole

Chemical Formula: $C_{27}H_{20}Cl_2FN_3O_2$; Molecular weight: 508.37; IR (cm^{-1}): 3045 (N-H); 2960 (C-H); 1625 (C=O); 1440 (C=N); 1622 (C=C); 1125 (C-O); 1012 (C-Br); 848 (C-Cl); 1098 (C-F); 1HNMR (ppm): δ 6.66-6.68 (d, 2H, Ar-Fluorine), 7.14-7.18 (d, 1H, Ar-Chlorine), 7.28-7.35 (m, 5H, Ar-H), 7.56-7.58 (d, 2H, Ar-Chlorine), 7.72-7.78 (d, 2H, Ar-Fluorine), 8.22 (s, 1H, Ar-H isoxazole ring), 10.97 (s, 1H, -Ar-H pyrazole ring); FAB Mass (m/z): 508.30.

PHARMACOLOGICAL EVALUATION:

Disk diffusion method:

The antibacterial activity of synthesized compounds **(PIA-1 to PIA-10)** was screened against each gram positive and gram-negative strains by disc diffusion method using sulfamethoxazole as standard drug. The data of antibacterial activity of synthesized derivatives was presented in Table 1 and 2.

Table 1: Antibacterial activity of synthesized quinazolinone derivatives against gram-positive bacteria

Compound	Zone of Inhibition (mm)			
	Gram positive bacteria			
	Bacillus subtilis		*Micrococlus luteus*	
Conc.	**50 µg/ml**	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**
PIA-1	13.12±0.3	14.10±0.8	12.52±0.2	17.62±0.6
PIA-2	8.42±0.8	9.22±0.4	9.31±0.3	11.42±0.8
PIA-3	11.72±0.3	13.15±0.4	13.28±0.5	15.32±0.2
PIA-4	11.22±0.6	13.62±0.3	11.42±0.8	15.22±0.5
PIA-5	10.22±0.7	12.52±0.2	10.14±0.5	13.32±0.7
PIA-6	8.52±0.5	9.22±0.4	6.22±0.5	7.15±0.5
PIA-7	9.22±0.3	10.62±0.6	7.42±0.7	8.54±0.4
PIA-8	7.62±0.8	8.02±0.7	8.31±0.2	10.12±0.4
PIA-9	6.22±0.2	7.52±0.5	5.52±0.3	6.02±0.3
PIA-10	4.42±0.3	6.46±0.4	6.06±0.5	7.22±0.7
Sulfamethoxazole (25µg/ml)	16.22±0.3	19.45±0.5	15.25±0.5	21.52±0.4

Table 2: Antibacterial activity of synthesized quinazolinone derivatives against gram-negative bacteria

COMPOUND	Zone of Inhibition			
	Gram Negative Bacteria			
	Pseudomonas Aeruginosa		*Escherichia Coli*	
	50 µg/ml	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**
PIA-1	14.15±0.5	16.22±0.3	15.32±0.8	17.62±0.7
PIA-2	7.22±0.6	9.14±0.7	7.13±0.4	8.56±0.3
PIA-3	12.12±0.3	14.52±0.7	12.52±0.2	14.22±0.5
PIA-4	12.03±0.5	13.37±0.6	13.42±0.3	15.17±0.5

PIA-5	7.52±0.7	9.42±0.9	8.52±0.4	9.52±0.2
PIA-6	6.62±0.4	8.12±0.8	7.07±0.3	8.17±0.7
PIA-7	4.42±0.3	6.46±0.4	6.06±0.5	7.22±0.7
PIA-8	6.12±0.3	6.14±0.4	6.02±0.2	6.14±0.3
PIA-9	6.52±0.6	6.15±0.7	6.14±0.4	6.16±0.8
PIA-10	9.42±0.8	11.32±0.4	10.32±0.7	12.62±0.3
Sulfamethoxazole (25µg/ml)	16.64±0.3	17.45±0.3	17.64±0.2	20.65±0.4

Antibacterial activity:

The Ten synthesized compounds (PIA-1 to PIA-10) were evaluated for the antibacterial screening and data obtained by the result stated that Isoxazole bearing pyrazole derivatives has shown the mild to best activity against tested gram positive and gram-negative organism's strains. The disc diffusion method is applied for the evaluated of the synthesized compounds (PIA-1 to PIA-10) and result data stated that compound PIA-1 shown the best activity as compared to standard antibacterial drug (Ciprofloxacin).

The four (04) compounds out of Ten (10) compounds have shown the antibacterial potential although the remains are failed to protect against bacterial infection. The data of antibacterial activity against the gram-positive bacteria recommended that among the synthesized compounds (PIA-1 to PIA-10), compound PIA-10, PIA-9 and PIA-8 have shown mild activity while as compound PIA-7, PIA-6 and PIA-2 shown moderate activity and compound PIA-1, PIA-3, PIA-4 and PIA-5 have shown better activity against gram positive bacteria (Table 1). The representation of zone of inhibition on gram positive and gram-negative bacterial strains was shown in Figure 1 & 2.

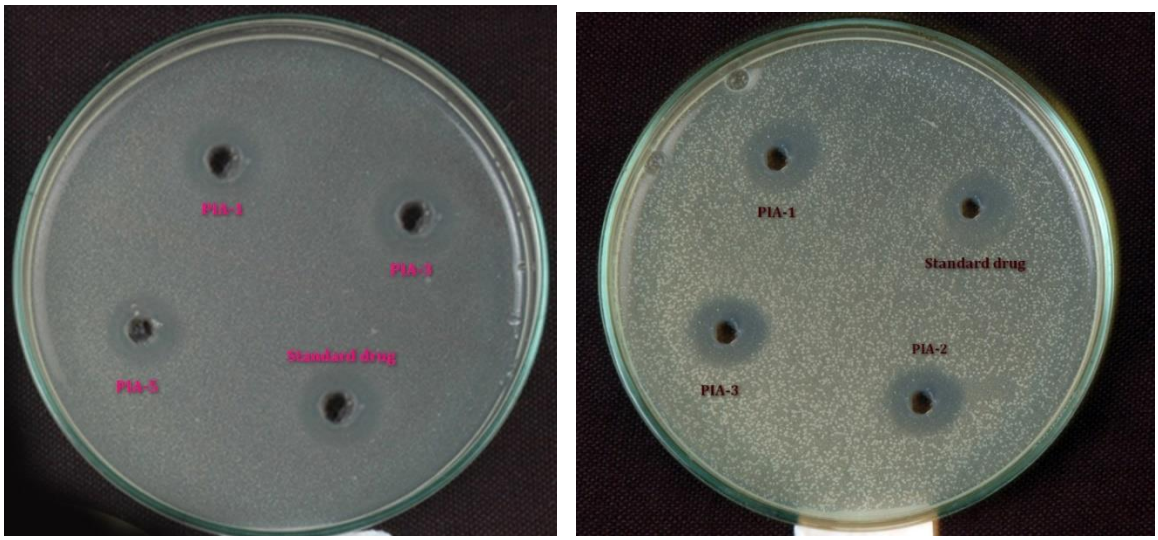


Fig. 1: The pictorial representation of Zone of inhibition of compound against gram-positive bacteria.

The compounds PIA-1 (13.12±0.3; 12.52±0.2), PIA-3 (11.72±0.3; 13.28±0.5); PIA-4 (11.22±0.6; 11.42±0.8), and PIA-5 (10.22±0.7; 10.14±0.5) have shown zone of inhibition (ZOI) in comparison to standard drug (Sulfamethoxazole, 16.22±0.3; 15.25±0.5) has shown good activity at 50µg concentration against gram-positive bacterial strains (*B. Subtilis* and *M. Luteus*) respectively, The compound PIA-1 (14.10±0.8; 17.62±0.6), PIA-4 (13.15±0.4; 15.32±0.2), PIA-4 (13.62±0.8; 15.22±0.3) and PIA-5 (10.62±0.6; 8.54±0.4) have shown ZOI in comparison to standard drug (Sulfamethoxazole , 19.45±0.5; 21.52±0.4) has shown good activity at 100µg concentration against gram-positive bacterial strains (*B. Subtilis* and *M. Luteus*).

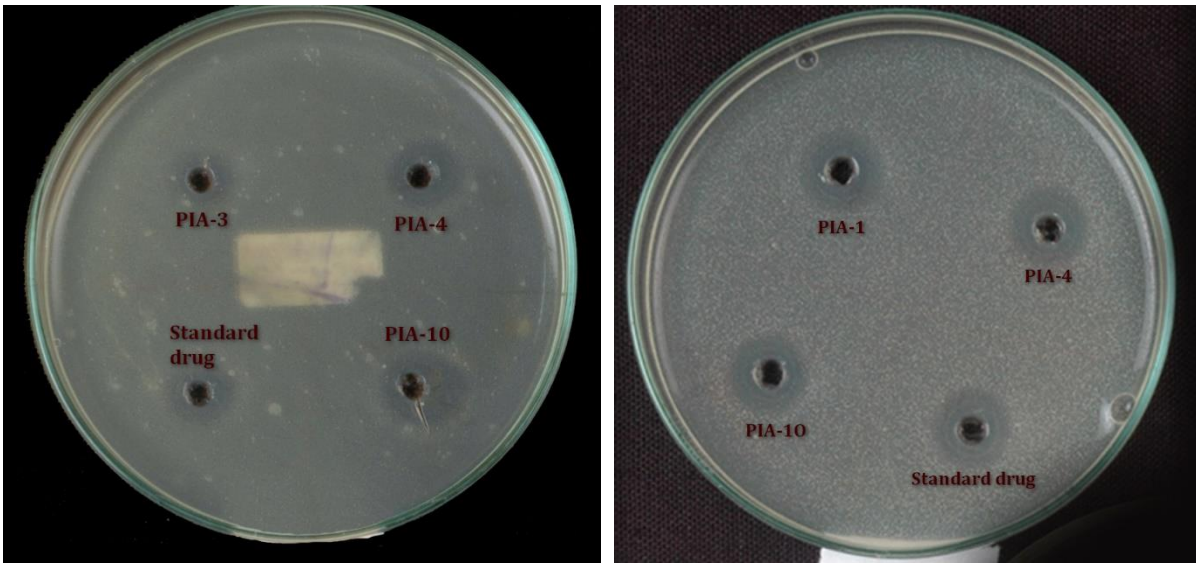


Fig. 2: The pictorial representation of Zone of inhibition of synthesized compounds against gram negative bacteria

The Data of antibacterial activity against the gram-negative bacterial strains suggested that among synthesized compounds (PIA-1 to PIA-10), compound PIA-9, PIA-8, PIA-7, and PIA-6 have shown mild activity while compound PIA-2, PIA-5 shown moderate activity and compound PIA-1, PIA-3, PIA-4 and PIA-10 have shown best activity against gram negative bacteria (Table 2).

DISCUSSION:

The antibacterial activity of synthesized compounds **(PIA-1 to PIA-10)** were screened against Gram-positive strains (*Bacillus subtilis* and *Micrococlus luteus*) and Gram-negative strains (*Pseudomonas aeruginosa* and *Escherichia coli*) by disc diffusion method using sulfamethoxazole as standard drug. The antibacterial evaluation of the synthesized compounds stated that Isoxazole-pyrazole derivatives (PIA-1 to PIA-10) exhibit considerable inhibitory effect on all bacteria strains at both 50 µg/ml and 100 µg/ml dosing levels. PIA-1, PIA-2, PIA-3, PIA-4 had the highest activity in gram positive bacterial stains and PIA-1, PIA-3, PIA-4 and PIA-10 have shown best activity against gram negative bacteria. These compounds have halogens on the ring, indicating that electron withdrawing groups play a favorable role in antibacterial action. Potent antibacterial action requires the presence of an electronegative group (Cl, F, NO_2).

CONCLUSION:

The goal of this thesis is to synthesize the dual moieties-based compounds includes the Isoxazole in pyrazole compound for searching the new antimicrobial agents. The spectral and qualitative analysis demonstrated the compounds has synthesized as according they designed. The antibacterial activity of the synthesized compounds (PIA-1, PIA-3, PIA-4 and PIA-5) was significantly similar or better than that of standard Drug used for comparison. These Isoxazole bearing pyrazole compounds have demonstrated potency as a superior antibacterial agent, although more in-vivo research on the efficacy and dosage regimen of the compounds is needed.

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Conflicts of Interest:

The author declares no conflicts of interest

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